Review Article

Molecular mechanisms of insulin resistance

T. S. PILLAY, M. W. MAKGOBA

Summary

This review discusses recent advances in understanding of the structure and function of the insulin receptor and insulin action, and how these relate to the clinical aspects of insulin resistance associated with non-insulin-dependent diabetes and other disorders. Improved understanding of the molecular basis of insulin resistance could ultimately lead to a better understanding of the causation of these conditions and the design of rational therapy to ameliorate them. Here, particular attention is devoted to the initial events that follow the binding of insulin to its receptor, including changes in insulin receptor phosphorylation. Receptor-mediated insulin resistance may be a consequence of various factors including increased serine/threonine phosphorylation of the receptor with decreased tyrosine phosphorylation, receptor densitisation, auto-antibodies to the receptor and inherited structural defects in the insulin receptor. Defects in insulin action could also arise at post-receptor events particularly glucose transport. Other circulating hormones, such as the newly characterised islet amyloid polypeptide (amylin), may also cause insulin resistance.

S Afr Med J 1991; **79:** 607-613.

A major abnormality in non-insulin-dependent diabetes (NIDDM) and some rare genetic syndromes is insulin resistance. Insulin is the principal hormone controlling blood glucose, and it achieves this function by interacting with specific receptors on cell surfaces mainly in liver, muscle and adipose tissue. The presence of insulin resistance in patients implies that a defect is present somewhere along the line of insulin action from receptor to the alteration of blood glucose. Hence, in order to elucidate the nature of this defect in diabetes mellitus, it is important to study the actions of insulin *in vitro* and *in vivo*. Because the binding of insulin to its receptor is the first step in the action of insulin, it is appropriate that we should attempt to understand the structure, function and regulation of the insulin receptor in our efforts to unravel the cause of this resistance to insulin. The insulin field is notable for its scientific landmarks over the last 6 decades. Insulin was among the first peptide hormones to be isolated;[1] the first protein to have its amino acid sequence determined;[2] the first peptide hormone to have its three-dimensional structure determined[3] and its gene cloned.[4] The insulin receptor was among the first peptide hormone receptors to be characterised and purified.[5] Despite these intensive efforts by many investigators, the molecular basis of insulin action remains largely a mystery in comparison with other hormone receptor systems. Research has been hampered by the temporally and biochemically divergent nature of the cellular events that follow the high-affinity binding of insulin to specific cell surface receptors in its principal target tissues. Very few of these events can be explained or unified by any of the well-defined mechanisms of signal transduction.

Division of Molecular Endocrinology, Department of Chemical Pathology, Royal Postgraduate Medical School, Hammersmith Hospital, London
T. S. PILLAY, M.B. CH.B., PH.D. (CANTAB.)
M. W. MAKGOBA, M.B. CH.B., D.PHIL. (OXON.), F.R.C.P.

Reprint requests to: Dr T. S. Pillay, Dept of Chemical Pathology, Royal Postgraduate Medical School, Du Cane Road, London W12 ONN, UK.
Accepted 24 Oct 1990.

Structure, function and regulation of the insulin receptor

Initial biochemical studies showed that the insulin receptor is an integral membrane glycoprotein composed of two subunits, α and β, linked to form a heterotetrameric β-α-α-β complex.[6] The β-subunit has intrinsic insulin-stimulated tyrosine kinase activity.[7] These findings were confirmed by the cloning of the complementary DNA (cDNA) and the consequent deduced amino acid sequence.[8,9] The insulin receptor gene is located on chromosome 19, spans more than 120 kilobases and has 22 exons (coding regions) interrupted by introns (intervening, non-coding sequences) of variable length.[10] Some of the exons code for discrete functional units (Fig. 1). The insulin receptor is synthesised as single polypeptide precursor that is predestined to form the mature receptor subunits, α and β (Fig. 1). The precursor is cleaved by processing enzymes to form two subunits, the 135 kD α subunit and a 95 kD β-subunit. These subunits are then assembled into a mature tetrameric complex linked by disulphide bonds and inserted into cell surface membranes (Fig. 1, Fig. 2). The α-subunits are located extracellularly and most of the β-subunits are intracellular. The α-subunit has a cysteine-rich domain (residues 155-312) that is also seen in the EGF (epidermal growth factor), IGF-I (insulin-like growth factor I) and LDL (low-density lipoprotein) receptors.[8,9] It exists as two isoforms that differ in the presence/absence of a 12-amino acid insert (Ebina[8] extension) (residues 718-729 according to the sequence of Ebina *et al.*[8] owing to alternative splicing of the insulin receptor mRNA transcript. Insulin binds to the α-subunit and triggers a conformational change that causes the β-subunit to autophosphorylate on tyrosine residues (Fig. 3). Several regions on the α-subunit have been implicated in the formation of the insulin binding site: (*i*) a region encoded for by exon 2 (residues 7-190) with phenylalanine 89 being necessary for high-affinity binding,[11] and (*ii*) residues 242-247 in the cysteine-rich domain.[12] The β-subunit has features in common with other tyrosine kinase oncogenes and serine/threonine protein kinases including ATP-binding and catalytic domains.[8,9] The insulin receptor is closely related to the IGF-I (somatomedin-C) receptor in its overall structural organisation, amino acid sequence and function.[13] The growth-promoting effects of insulin may be mediated by the IGF-I receptor. Autophosphorylation of the β-subunit activates the receptor towards

phosphorylating other substrates.[14] The events linking this autophosphorylation reaction with the eventual cellular effects of insulin are somewhat obscure and the subject of intensive research. Evidence derived from genetically engineered mutant receptors suggests that the tyrosine kinase activity and autophosphorylation of the receptor are important for insulin action. Removal of the ATP-binding site or some of the major autophosphorylation sites by site-directed mutagenesis abolishes most of the effects of insulin.[15,16]

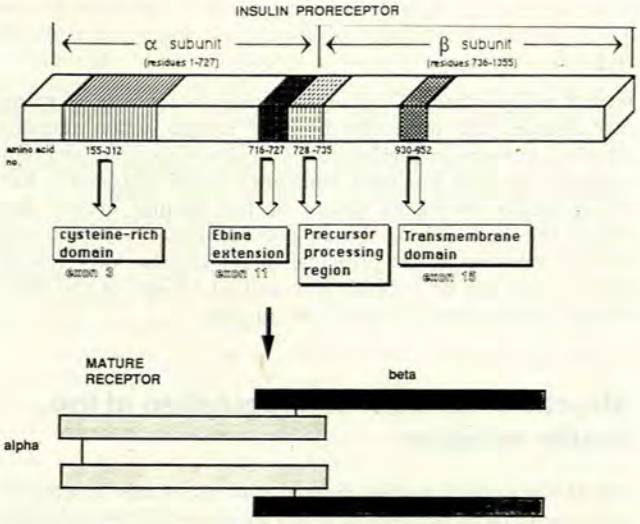

Fig. 1. Biosynthesis of the insulin receptor. Like insulin, the receptor is synthesised as a single polypeptide precursor, the proreceptor. The proreceptor is then cleaved into α- and β-subunits and assembled to form the minimal functional unit, the heterotetramer. Some of the exons of the insulin receptor gene code for discrete functional units. Exon 11 may be spliced out from messenger RNA to generate two insulin receptor isoforms that differ only in the presence or absence of the region coded for by exon 11.

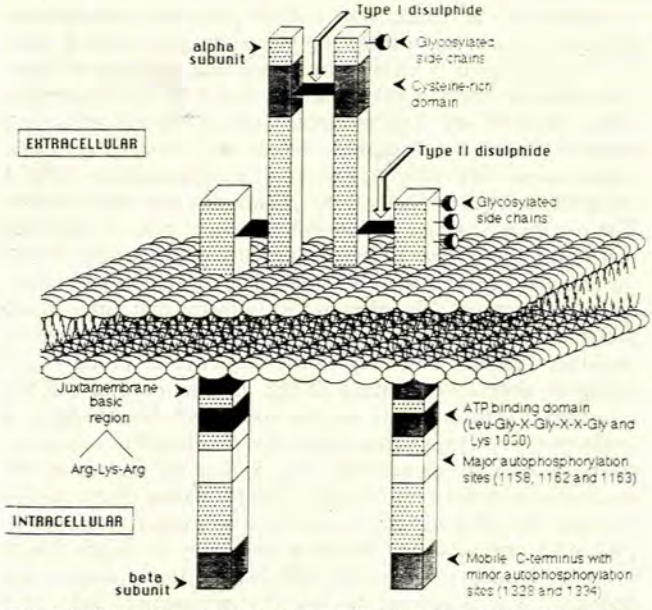

Fig. 2. Schematic model of the insulin receptor. The 3-D structure of the receptor is as yet unknown and hence this model is highly idealised. The receptor is represented as a heterotetrameric complex. The α-subunit is located extracellularly. The β-subunit traverses the cell membrane with most of the β-subunit being located intracellularly. Both α- and β-subunits are substantially glycosylated in their extracellular portions. The juxtamembrane region (Arg-Lys-Arg) may function in signal transduction by interacting with phospholipids.

Transmembrane signalling and insulin receptor phosphorylation

Two major hypotheses are commonly invoked to explain the nature of insulin receptor signalling. Firstly, the initiation of a phosphorylation/dephosphorylation cascade by the receptor kinase with the participation of various kinases and/or phosphatases. Alternatively or concurrently, alteration of receptor conformation by autophosphorylation to a state that facilitates its non-covalent interaction with other effector systems and/or generates a second messenger[17] in a manner reminiscent of cyclic nucleotides. These distinct pathways could either diverge or act synergistically to co-ordinate cellular responses.

The rapid insulin-stimulated autophosphorylation of the insulin receptor on tyrosine residues 1158, 1162, 1163, 1328 and 1344[18-20] is followed by a slower phosphorylation on serine and threonine residues of the C-terminus, particularly serines 1305, 1306 and threonine 1348[21-23] (Fig. 3). This second serine/threonine phosphorylation reaction is mediated by another distinct protein kinase, an insulin-activated receptor serine kinase (IRSK),[22,23] that is closely associated with the insulin receptor. The activation of the IRSK is dependent upon the initial tyrosine autophosphorylation and hence it has been hypothesised that the insulin receptor directly phosphorylates the IRSK on tyrosine. This leads to activation of IRSK and the IRSK phosphorylates the insulin receptor in a 'retrograde' manner. It is known that insulin action is associated with the changes in serine phosphorylation of a number of cellular proteins and hence this IRSK may also then initiate a cascade of phosphorylation reactions that results in the effects of insulin.

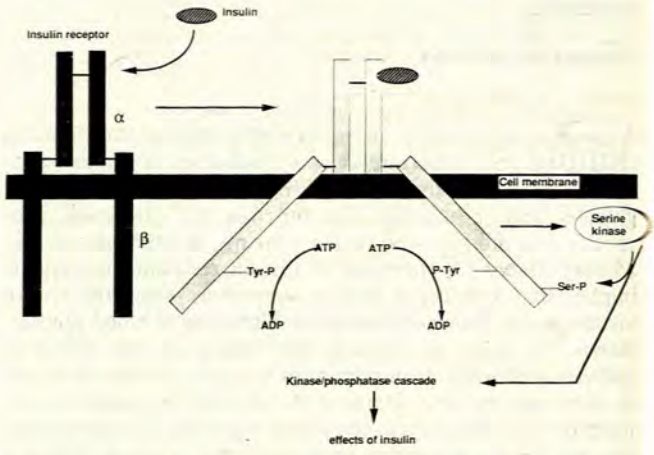

Fig. 3. Insulin-stimulated phosphorylation of the insulin receptor. The binding of insulin to the α-subunit stimulates the phosphorylation of the β-subunit on tyrosine residues. In intact cells, tyrosine autophosphorylation of the β-subunit is followed by phosphorylation of the β-subunit on serine/threonine residues in the C-terminus. This serine/threonine phosphorylation is mediated by a closely associated enzyme, the insulin receptor serine kinase (IRSK).

Regulation of the insulin receptor by serine/threonine phosphorylation

What is the function of the second phosphorylation reaction (serine/threonine) that occurs on the insulin receptor? No functional effects of this reaction have been demonstrated, but in other situations increased serine/threonine phosphorylation in response to activators of other serine/threonine kinases results in an attenuation of the tyrosine autophosphorylation and the tyrosine kinase activity. For example, the tumour-

promoting phorbol esters that activate protein kinase C result in increased serine/threonine phosphorylation of the insulin receptor with accompanying decreases in tyrosine phosphorylation and kinase activity.[24] When cellular levels of protein kinase C are decreased a potentiation of the insulin-stimulated tyrosine autophosphorylation occurs, suggesting that protein kinase C exerts a tonic inhibitory effect on the insulin receptor.[25] Furthermore, elevation of intracellular cyclic AMP, which stimulates the cyclic AMP-dependent protein kinase, also results in increased serine/threonine phosphorylation with a diminution of the tyrosine autophosphorylation.[26] These studies imply that the phosphorylation of the insulin receptor on serine/threonine residues may be a mechanism for negatively modulating the insulin receptor and consequently may be a mechanism of insulin resistance. By inference then, the insulin-stimulated serine/threonine phosphorylation by the IRSK is possibly a negative feedback mechanism whereby other receptors may be prevented from undergoing further activation. In this way, it is possible that the insulin receptor, in common with the β-adrenergic receptor,[27] limits its own signal transduction by simultaneously activating a receptor-specific serine/threonine kinase.

Cellular effects of insulin

How do the effects of insulin manifest? (for a review see references 28, 29, 30). The initial events (within seconds) include autophosphorylation of the receptor β-subunit with activation of the intrinsic tyrosine-specific protein kinase. The rapid phosphorylation on tyrosine residues is followed by a slower serine/threonine phosphorylation of the β-subunit. Also prominent in minutes are the changes in activity and phosphorylation states of numerous cellular proteins (Fig. 4). Physiologically, insulin lowers blood glucose. At the cellular level this is the result of the translocation of glucose carriers from an abundant intracellular pool to the cell surface. Insulin also causes the internalisation of its receptor and the hormone-receptor complex enters the cell by endocytosis. It is then either targeted for degradation or for recycling back to the cell surface. Elevated circulating levels of insulin increase the rate of receptor internalisation thus decreasing the numbers of receptors on the cell surface — a phenomenon referred to as downregulation. The longer-term effects of insulin include modulation of DNA and RNA synthesis and increased cell growth.

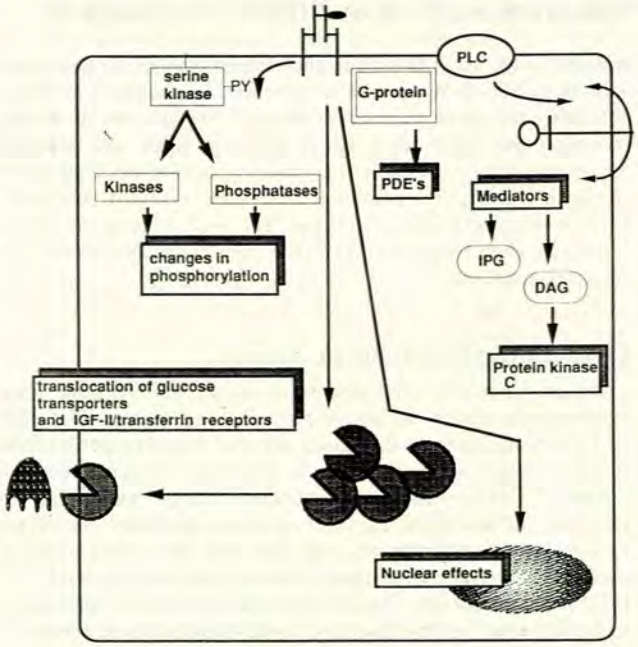

Fig. 4. Cellular effects of insulin. In addition to stimulating phosphorylation of the insulin receptor, insulin also provokes changes in the phosphorylation states of numerous intracellular enzymes. The translocation of glucose transporter vesicles to the cell surface may also be a phosphorylation-linked process. Other effects include the lowering of cyclic AMP levels via the activation of phosphodiesterases (PDE) and the generation of inositol phosphoglycan (IPG) mediators and diacylglycerol (DAG) from the glycosylphosphatidylinositol (glycosyl-PI) anchor. For simplicity the anchor is positioned on the inside of the cell. The inositol glycan mediator affects the activities of several enzymes including puruvate dehydrogenase, adenylate cyclase and acetyl-coA carboxylase. Over a longer period insulin also modulates nucleic acid synthesis, protein synthesis and cell growth.

Mechanisms of insulin resistance

Insulin resistance is said to occur when a physiological concentration of insulin results in a suboptimal effect. This decreased effect of insulin may be manifested as a rightward shift of the dose-response curve or a decrease in the maximal response. The shift in the dose-response curve is referred to as *decreased sensitivity* because more hormone is needed to produce the same effect, while the decrease in maximal response is called *decreased responsiveness*.[31] Decreased sensitivity implies a change in receptor number or affinity, while decreased responsiveness implies a change in a rate-limiting step, usually at a post-receptor level. From a clinical standpoint this distinction is important because decreased sensitivity can be overcome by increasing the dose of the hormone, whereas with decreased responsiveness it may not be possible to do this. Aetiologically, insulin resistance may be the consequence of circulating antagonists or target tissue defects (Fig. 5). Clinical conditions in which circulating antagonists of insulin are the principal cause of hyperglycaemia/insulin resistance (i.e. increased catecholamines, glucocorticoid excess, acromegaly) are not discussed in this review as these more commonly manifest as glucose intolerance. Cellular mechanisms of insulin resistance in these conditions are unclear owing to the fact that they have not been investigated in depth in patients or in cellular models. Target tissue defects may arise at the receptor-binding phase or the post-binding phase of insulin action.

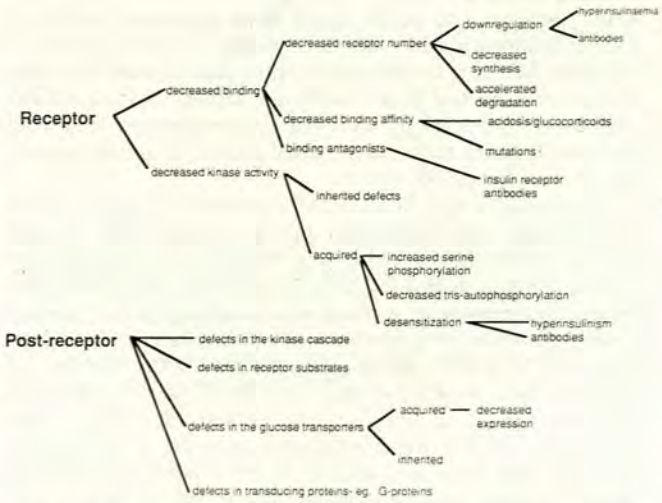

Fig. 5. Mechanisms of receptor and post-receptor-mediated insulin resistance. The mechanisms of post-receptor-mediated insulin resistance are as yet poorly defined as very little is known about the nature of the post-receptor pathways.

Insulin resistance in NIDDM and obesity

Resistance of target tissues to the actions of insulin is a central feature of NIDDM and is also observed in obesity.[32-34] Obese patients commonly show a decrease in the number of insulin receptors as a result of the prevailing hyperinsulinaemia (downregulation). Weight loss results in a reversal of these changes. Obesity is a common finding in NIDDM and hence patients with NIDDM also show decreased receptor numbers. However, even some lean NIDDM patients show decreases in receptor numbers.

Defects in the receptor kinase

Apart from the changes in receptor numbers *in vitro*, euglycaemic clamp studies *in vitro* indicate that in NIDDM and in obesity there is decreased sensitivity and responsiveness implying the existence of both receptor and post-receptor defects.[32-34] The possibility that these changes may be a consequence of an impairment of receptor-mediated signalling, i.e. its tyrosine kinase activity, has led to investigations of receptor kinase activity in both humans and animal models of NIDDM and obesity. Receptor kinase activity has been found to be impaired in erythrocytes,[35] liver[36] and adipose tissue[37-40] from obese NIDDM patients. Adipocytes from obese non-diabetics showed insulin-stimulated kinase activity comparable to that of normal controls, while obese diabetics showed a 50% reduction in kinase activity.[40] Although obese non-diabetics did not show the same defects,[40] the defect in obese diabetics was reversed by weight reduction. Skeletal muscle is quantitatively the most important target tissue for insulin in that it has a principal role in insulin-stimulated glucose disposal.[41] However, skeletal muscle showed slightly different results in that kinase activity was impaired by obesity *per se*, with or without NIDDM.[42,43] In contrast, autophosphorylation of muscle receptors from normal, obese and obese diabetic subjects was the same. The IGF-I receptor was not affected and the defect appeared to be specific for the insulin receptor.[44] Decreased receptor kinase activity is also seen in animal models of obesity[45,46] but not in other insulin-resistant states.[47] The mechanisms of decreased kinase activity observed in all these studies is uncertain. Tryptic peptide mapping of insulin receptors from diabetic skeletal muscle showed a decreased amount of the tris-phosphorylated peptides that reflects phosphorylation of tyrosine residues 1158, 1162 and 1163 and maximal kinase activation.[48] In adipose tissue decreased kinase activity appeared to be the result of an increased number of autophosphorylation-incompetent receptors.[49] Serine/threonine phosphorylation of the insulin receptor may regulate the autophosphorylation and hence the kinase activity of these insulin receptors. In addition, increased internalisation following hyperinsulinaemia may lead to partial proteolysis of the receptor and a loss of kinase activity.[50]

The resistance seen in NIDDM is probably of multifactorial origin. Numerous factors may operate to create the clinical features of insulin resistance (Fig. 6). Hyperinsulinaemia *per se*, in addition to decreasing receptor numbers, also desensitises the insulin receptor as a result of uncoupling of the tyrosine kinase activation from insulin binding.[51] Hence, a given concentration of insulin action acting on a similar number of receptors fails to achieve the same level of tyrosine autophosphorylation, kinase activity or cellular effects. The mechanism of this hyperinsulinism-induced uncoupling is unknown but does not appear to be related to increased serine/threonine phosphorylation of the insulin receptor in cellular models.[51] The initiating defect in the pathogenesis of insulin resistance in NIDDM is not known, but there appear to be interrelated events. Chronically elevated insulin levels lead to a decrease in the number of cell surface receptors (downregulation). This decrease in cell surface receptors is accompanied by an attenuation of the insulin-stimulated tyrosine kinase activity and an increase in the number of autophosphorylation-incompetent receptors. Additionally, this is associated with a decrease in the tris-phosphorylated state that is required for maximal activation of the insulin receptor kinase. Studies are currently in progress to determine whether this defective tyrosine kinase activity is associated with increased serine/threonine phosphorylation of the receptor in humans and animal models of insulin resistance.

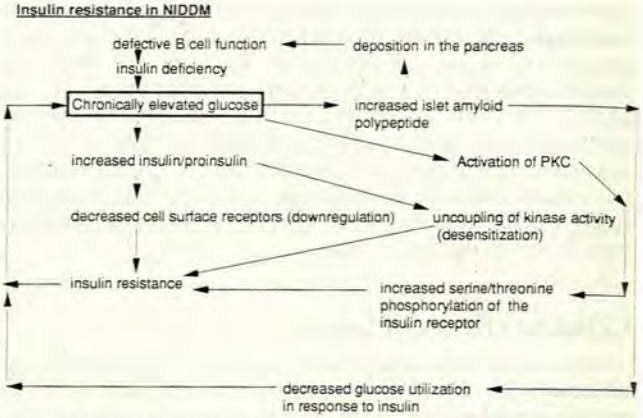

Fig. 6. Mechanisms of insulin resistance in NIDDM. Insulin resistance in NIDDM appears to be the result of numerous factors resulting in both receptor and post-receptor defects (PKC protein kinase C).

Islet amyloid polypeptide

Another factor that may operate in this scenario is the presence of elevated levels of islet amyloid polypeptide/amylin.[52] This recently characterised hormone is co-secreted with insulin from the B cells of the pancreas.[52] Its precise function is unknown. However, increased deposition of this hormone is seen in the pancreata of patients with diabetes mellitus.[52] In cells and in animal studies, it has been reported to cause insulin resistance and glucose intolerance.[53,54] NIDDM is associated with increased levels of this hormone (R. Seth, A. Donaldson and M. W. Makgoba — unpublished data), and it has been hypothesised that this may also be responsible for the insulin resistance seen in NIDDM.[52]

Glucose transporter defects

As increased glucose uptake is a major action of insulin, it is possible that defects may arise at the level of the glucose transporters. Specific glucose carrier proteins facilitate the transport of glucose into cells. These glucose transporters are derived from a multigene family of related proteins that are expressed in a well-defined tissue-specific manner.[55] Five such facilitated transporters (designated GLUT1-5 according to the nomenclature of Bell *et al.*[55]) have been identified from cDNA clones.[55] In the context of insulin action, the GLUT4 isoform found in muscle and fat is the most important and mediates the insulin-stimulated uptake of glucose that occurs postprandially.[56-59] The GLUT2 (liver) isoform is found in the pancreatic B cells and may be responsible for sensing changes in blood glucose.[60] Hence defects in these two transporter proteins could contribute to the pathophysiology of diabetes mellitus. Other studies suggest that genetic variations in the GLUT1 isoform may be associated with an increased risk of NIDDM,[61] but this has not been confirmed.[62,63]

Adipocytes and muscle cells from diabetic animals show a marked decrease in insulin-stimulated glucose uptake owing to a decrease in the number of glucose transporters at the cell surface.[64,65] Impaired insulin action in adipocytes from diabetic animals is associated with a decrease in the level of expression of the insulin-stimulated GLUT4 isoform. In diabetic humans, studies on adipocytes have described a decrease in whole cell glucose transport as a result of defects in the number and function of glucose transporters.[55]

Extreme insulin resistance

In humans extreme or severe insulin resistance is seen in the context of several congenital syndromes including the type A syndrome, leprechaunism and lipo-atrophic diabetes (for reviews see references 66 and 67). Although distinctly rare and diverse, studies in patients with these conditions have provided some insight into the structure and function of the insulin receptor. This extreme insulin resistance may be the result of markedly decreased insulin binding and/or receptor kinase activity.[68–71] The decrease in binding is usually the result of decreased numbers of cell surface receptors. The autophosphorylation and kinase activity of monocyte insulin receptors from a patient with the type A syndrome[72] was decreased by 90% in comparision with normal controls. This occurred in the presence of normal insulin binding affinity. Erythrocytes and fibroblasts from other type A patients showed similar, though less severe, impairment of kinase activity.[68,69] Insulin receptors from fibroblasts, hepatocytes and lymphocytes of a lipodystrophic patient also showed decreased kinase activity.[71] Although these studies support an association between defects in kinase activity and severe insulin resistance, impaired kinase activity is not observed in all subjects with extreme insulin resistance.[72–74]

The availability of the human insulin receptor cDNA coupled with the polymerase chain reaction has made it possible to examine the nucleotide sequence of the insulin receptor in several such patients. Mutations have been identified in both subunits of the insulin receptor (reviewed in reference 75). Some of these (e.g. $Arg^{909}\rightarrow$ stop codon) (residue numbers according to the deduced sequence of Ebina *et al.*[8]) affect the synthesis of the insulin receptor mRNA and manifest as a decreased number of cell surface receptors in binding studies. Others ($Phe^{382}\rightarrow$ Val) affect the transport of the receptor to the cell surface after synthesis. The first mutation described took the form of $Arg^{747}\rightarrow$ Ser within the tetrabasic cleavage region. This resulted in uncleaved precursor with decreased binding affinity being inserted into the plasma membrane. Further mutations decrease the binding function of the receptor ($Leu^{233}\rightarrow$ Pro) or its kinase activity ($Gly^{1020}\rightarrow$ Vall; $Trp^{1212}\rightarrow$ Ser). One particular mutation ($Lys^{460}\rightarrow$ Glu) actually increases the binding affinity of the receptor. However, because this results in accelerated downregulation and preferential degradation of the insulin receptor, insulin resistance occurs. In general, mutations in the extracellular regions of the receptor affect the binding of insulin whereas intracellular mutations result in decreased kinase activity.

The contribution of receptor gene mutations to the insulin resistance of NIDDM is as yet unclear, although studies are underway to evaluate this aspect.[76] It is possible that some receptor gene mutations may not be severe or extensive enough to cause a full-blown syndrome of extreme insulin resistance, and may instead manifest in the milder insulin resistance of NIDDM.

Auto-antibodies

Auto-antibodies to the insulin receptor may also cause insulin resistance as part of the type B syndrome of extreme insulin resistance.[77] Most of these antibodies generally inhibit insulin binding, while some are insulinomimetic. These antibodies may also cause downregulation of the insulin receptor and hence decrease insulin receptor numbers at the cell surface. Some patients with NIDDM[78] or newly diagnosed IDDM[79] develop low titres of auto-antibodies that arise partly as anti-idiotypes of anti-insulin antibodies.[80,81]

Hybrid receptors

Insulin and IGF-I receptor α-β-subunit dimers may associate to form 'hybrid' receptors (Fig. 7). Evidence for the existence of these has been provided by Soos and Siddle[82] and Moxham *et al.*[83] These partially explain why insulin and IGF-I can mimic each other's effects on cells.[84] The growth-promoting effects of insulin, seen classically in fetal macrosomia following diabetic pregnancy, may largely be due to the possibility that insulin can stimulate growth-promoting pathways by acting through these hybrid receptors. Furthermore, it indicates that IGF-II may also exert insulin/IGF-I-like effects, presumably by acting through such receptors as the deduced structure of the IGF-II receptor indicates that is unlikely to have any signalling function. Thus hybrid receptors could explain the occurrence of tumour-associated hypoglycaemia ('specificity spillover phenomenon')[85] seen with non-islet cell tumours that produce IGF-II.[86,87] Whittaker *et al.*[88] have suggested that the hybrid receptors may also explain the effect of negative-dominant insulin receptor mutations in patients with insulin-resistant syndromes, i.e. why patients with mutant receptors and who are simple hyterozygotes should show evidence of insulin resistance. Because the minimal functional unit of the insulin receptor is a heterotetramer, some mutant α-β-dimers may associate with normal α-β-dimers and impair the signalling functions of the receptor.

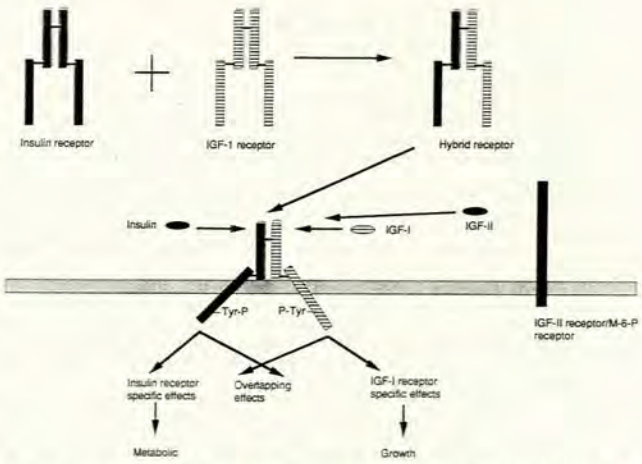

Fig. 7. Hybrid insulin/IGF-I receptors. IGF-I receptors are remarkably similar to insulin receptors in their gross structural organisation, size, amino acid sequence and function. Receptor subunit dimers may dissociate under suitable redox conditions and reassemble with dimers from the heterologous receptor. Alternatively, the assembly of hybrids may occur during synthesis of the respective receptors. IGF-II mediates its biological effects through its heterologous relatives.

Conclusions

While the precise molecular mechanism of insulin action continues to be a mystery, various pieces are slowly being added to the puzzle. Over the next few years much attention will be devoted towards defining the immediate post-receptor

events. It will be important to elucidate the links between receptor activation and glucose transport. This will involve isolation of the enzymes involved and eventual molecular cloning. Particular effort is being devoted towards isolating cellular substrates for the insulin receptor as it is essentially an enzyme. Recent advances in recombinant DNA technology, particularly the polymerase chain reaction, have made it possible to investigate patients for mutations in the insulin receptor or the glucose transporters. It is by attempting to solve the questions regarding insulin and insulin receptor function at the molecular level that it will be possible to understand the pathophysiology of insulin resistance.

T.S.P. was a recipient of the Emanuel Bradlow Scholarship from the Bradlow Foundation. Parts of this work were supported by a Hammersmith and Queen Charlottes Special Health Authority grant for locally organised research. M.W.M. is supported by the British Medical Research Council.

Addendum

It has recently been demonstrated that fibroblasts from a patient with insulin resistance and NIDDM produce a glycoprotein inhibitor of insulin receptor kinase activity (Sbracchia, P, Goodman PA, Maddux BA *et al. Diabetes* 1991; **40:** 295-299).

REFERENCES

1. Banting FG, Best CH. The internal secretion of the pancreas. *J lab Clin Med* 1922; **7:** 251-252.
2. Sanger F. Chemistry of insulin. *Science* 1959; **129:** 1340-1343.
3. Blundell TL, Dodson GG, Hodgkin DC, Mercola DA. Insulin: the structure in the crystal and its reflection in chemistry. *Adv Protein Chem* 1972; **26:** 279-284.
4. Bell GI, Swain WF, Pictet R, Cordell B, Goodman HM, Rutter WJ. Nucleotide sequence of a cDNA clone encoding human preproinsulin. *Nature* 1979; **282:** 525-527.
5. Cuatrecasas P. Isolation of the insulin receptor of liver and fat cell membranes. *Proc Natl Acad Sci USA* 1972; **69:** 318-322.
6. Massague J, Pilch PF, Czech MP. Electrophoretic resolution of three major insulin receptor structures with unique subunit stoichiometries. *Proc Natl Acad Sci USA* 1980; **77:** 7137-7141.
7. Kasuga M, Karlsson FA, Kahn CR. Insulin stimulates the phosphorylation of the 95,000-dalton subunit of its own receptor. *Science* 1982; **215:** 185-186.
8. Ebina Y, Ellis L, Jarnagin K *et al.* The human insulin receptor cDNA: the structural basis for hormone-activated transmembrane signalling. *Cell* 1985; **40:** 747-758.
9. Ullrich A, Bell JR, Chen EY *et al.* Human insulin receptor and its relationship to the tyrosine kinase family of oncogenes. *Nature* 1985; **313:** 756-761.
10. Seino S, Seino M, Nishi S, Bell GI. Structure of the human insulin receptor gene and characterization of its promoter. *Proc Natl Acad Sci USA* 1989; **86:** 114-118.
11. De Meyts P, Gu J-I, Shymko RM, Kaplan BE, Bell GI, Whittaker J. Identification of a ligand binding region of the insulin receptor encoded by the second exon of the gene. *Mol Endocrinol* 1990; **4:** 409-416.
12. Waugh SM, DiBella EE, Pilch PF. Isolation of a proteolytically derived domain of the insulin receptor containing the major site of cross-linking/binding. *Biochemistry* 1989; **28:** 3448-3455.
13. Ullrich A, Gray A, Tam AW *et al.* Insulin like growth factor I receptor primary structure: comparison with insulin receptor suggests structural determinants that define functional specificity. *EMBO J* 1986; **5:** 2503-2512.
14. Yu KT, Czech MP. Tyrosine phosphorylation of the insulin receptor beta subunit activates the receptor-associated tyrosine kinase activity. *J Biol Chem* 1984; **259:** 5277-5286.
15. Ellis L, Clauser E, Morgan DO, Edery M, Roth RA, Rutter WJ. Replacement of insulin receptor tyrosine residues 1162 and 1163 compromises insulin-stimulated kinase activity and uptake of 2-deoxyglucose. *Cell* 1986; **45:** 721-732.
16. Ebina Y, Araki E, Taira M *et al.* Replacement of lysine residue 1030 in the putative ATP-binding region of the insulin receptor abolishes insulin- and antibody-stimulated glucose uptake and receptor kinase activity. *Proc Natl Acad Sci USA* 1987; **84:** 704-708.
17. Saltiel A. Second messengers of insulin action. *Diabetes Care* 1990; **13:** 244-236.
18. Tavare JM, Denton RM. Studies on the autophosphorylation of the insulin receptor from human placenta: analysis of the sites phosphorylated by two-dimensional phosphopeptide mapping. *Biochem J* 1988; **252:** 607-615.
19. Tornqvist HE, Gunsalus JR, Nemenoff RA, Frackelton AR, Pierce MW, Avruch J. Identification of the insulin receptor tyrosine residues undergoing insulin-stimulated phosphorylation in intact rat hepatoma cells. *J Biol Chem* 1987; **263:** 350-359.
20. White MF, Shoelson SE, Keutmann H, Kahn CR. A cascade of tyrosine autophosphorylation in the beta-subunit activates the phosphotransferase of the insulin receptor. *J Biol Chem* 1988; **263:** 2969-2980.
21. Lewis RE, Cao L, Perregaux D, Czech MP. Threonine 1336 of the human insulin receptor is a major target for protein kinase C. *Biochemistry* 1990; **29:** 1807-1813.
22. Lewis RE, Wu GP, MacDonald RG, Czech MP. Insulin-sensitive phosphorylation of serine 1293/1294 on the human insulin receptor by a tightly associated serine kinase. *J Biol Chem* 1990; **265:** 947-954.
23. Pillay TS. Regulation of insulin receptors by serine/threonine phosphorylation. Ph.D. thesis, University of Cambridge, 1990.
24. Takayama S, White MF, Kahn CR. Phorbol ester-induced serine phosphorylation of the insulin receptor decreases its tyrosine kinase activity. *J Biol Chem* 1988; **263:** 3440-3447.
25. Pillay TS, Whittaker J, Siddle K. Phorbol ester-induced downregulation of protein kinase C potentiates insulin receptor tyrosine autophosphorylation: evidence for a major constitutive role in receptor regulation. *Biochem Soc Trans* 1990; **18:** 494-495.
26. Stadtmauer L, Rosen OM. Increasing the cAMP content of IM-9 cells alters the phosphorylation state and protein kinase activity of the insulin receptor. *J Biol Chem* 1986; **261:** 3402-3407.
27. Benovic JD, Strasser RH, Caron MG, Lefkowitz RJ. Beta adrenergic receptor kinase: identification of a novel protein kinase that phosphorylates the agonist occupied form of the receptor. *Proc Natl Acad Sci USA* 1986; **83:** 2797-2801.
28. Zick Y. The insulin receptor: structure and function. *CRC Crit Rev Biochem Mol Biol* 1989; **24:** 217-269.
29. Rosen OM. After insulin binds. *Science* 1987; **237:** 1452-1458.
30. Houslay MD, Siddle K. Molecular basis of insulin receptor function. *Br Med Bull* 1989; **45:** 264-284.
31. Kahn CR. Insulin resistance, insulin insensitivity and insulin unresponsiveness: a necessary distinction. *Metabolism* 1978; **27:** suppl 2, 1893-1902.
32. Koltermann OG, Gray R, Griffin J *et al.* Receptor and post-receptor defects contribute to the insulin resistance in non-insulin-dependent diabetes mellitus. *J Clin Invest* 1981; **68:** 957-969.
33. Truglia JA, Livingston JN, Lockwood DH. Insulin resistance: receptor and post-binding defects in human obesity and non-insulin dependent diabetes mellitus. *Am J Med* 1985; **79:** suppl 2B, 13-22.
34. Rizza RA, Mandarino LJ, Gerich JE. Mechanism and significance of insulin resistance in non-insulin-dependent diabetes mellitus. *Diabetes* 1981; **30:** 990-995.
35. Comi RJ, Grunberger G, Gorden P. Relationship of insulin binding and insulin-stimulated tyrosine kinase activity is altered in type II diabetes. *J Clin Invest* 1987; **79:** 453-462.
36. Caro JF, Ittoop O, Pories WJ *et al.* Studies on the mechanism of insulin resistance in the liver from humans with non-insulin-dependent diabetes: insulin action and binding in isolated hepatocytes, insulin receptor struture, and kinase activity. *J Clin Invest* 1986; **78:** 249-258.
37. Sinha MK, Pories WJ, Flickinger EG, Meelheim D, Caro JF. Insulin-receptor kinase activity of adipose tissue from morbidly obese humans with and without NIDDM. *Diabetes* 1987; **36:** 620-625.
38. Takayama S, Kahn CR, Kubo K, Foley JE. Alterations in insulin receptor autophosphorylation in insulin resistance: correlation with altered sensitivity to glucose transport and antilipolysis to insulin. *J Clin Endocrinol Metab* 1988; **66:** 992-999.
39. Freidenberg GR, Henry RR, Klein HH, Reichart DR, Olefsky JM. Decreased kinase activity of insulin receptors from adipocytes of non-insulin-dependent diabetic subjects. *J Clin Invest* 1987; **79:** 240-250.
40. Freidenberg GR, Reichart D, Olefsky JM, Henry RR. Reversibility of defective adipocyte insulin receptor kinase activity in non-insulin-dependent diabetes mellitus: effect of weight loss. *J Clin Invest* 1988; **82:** 1398-1406.
41. Olefsky JM. Insulin resistance and insulin action: an *in vitro* and *in vivo* perspective. *Diabetes* 1981; **30:** 148-162.
42. Caro JF, Sinha MK, Raju SM *et al.* Insulin receptor kinase in human skeletal muscle from obese subjects with and without noninsulin dependent diabetes. *J Clin Invest* 1987; **79:** 1330-1337.
43. Arner P, Pollare T, Lithell H, Livingston JN. Defective insulin receptor tyrosine kinase in human skeletal muscle in obesity and type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 1987; **30:** 437-440.
44. Livingston JN, Pollare T, Lithell H, Arner P. Characterization of insulin-like growth factor I receptor in skeletal muscles of normal and insulin-resistant subjects. *Diabetologia* 1988; **31:** 871-877.
45. Le Marchand-Brustel Y, Gremeaux T, Ballotti R, Van Obberghen E. Insulin receptor tyrosine kinase is defective in skeletal muscle of insulin resistant obese mice. *Nature* 1985; **315:** 676-679.
46. Shargill NS, Tatoyan A, El-Refai MF, Pleta M, Chan TM. Impaired insulin receptor phosphorylation in skeletal muscle membranes of db/db mice: the use of a novel skeletal muscle membrane preparation to compare insulin binding and stimulation of receptor phosphorylation. *Biochem Biophys Res Commun* 1986; **137:** 286-294.
47. Truglia JA, Hayes GR, Lockwood DH. Intact adipocyte insulin-receptor phosphorylation and *in vitro* tyrosine kinase activity in animal models of insulin resistance. *Diabetes* 1988; **37:** 147-158.
48. Obermaier-Kusser B, White MF, Pongratz DE *et al.* A defective intramolecular autoactivation cascade may cause the reduced kinase activity of the skeletal muscle insulin receptor from patients with non-insulin-dependent diabetes mellitus. *J Biol Chem* 1989; **264:** 9497-9504.
49. Brillon DJ, Freidenberg GR, Henry RR, Olefsky JM. Mechanism of defective insulin-receptor kinase activity in NIDDM: evidence of two receptor populations. *Diabetes* 1989; **38:** 397-403.
50. O'Hare T, Pilch PF. Separation and characterization of thee insulin receptor species that differ in subunit composition. *Biochemistry* 1988; **27:** 5693-5700.
51. Treadway JL, Whittaker J, Pessin J. Regulation of insulin receptor kinase by hyperinsulinism. *J Biol Chem* 1989; **264:** 15136-15143.
52. Johnson KH, O'Brien TD, Betsholz C, Westermark P. Islet amyloid, islet-amyloid polypeptide, and diabetes mellitus. *N Engl J Med* 1989; **321:** 513-518.
53. Leighton B, Cooper GJ. Pancreatic amylin and calcitonin gene-related peptide cause resistance to insulin in skeletal muscle *in vitro*. *Nature* 1988; **335:** 632-635.
54. Cooper GJ, Leighton B, Dimitriadis GD *et al.* Amylin found in amyloid deposits in human type 2 diabetes mellitus may be a hormone that regulates

glycogen metabolism in skeletal muscle. *Proc Natl Acad Sci USA* 1988; **85:** 7763-7766.
55. Bell GI, Kayano T, Buse JB *et al.* Molecular biology of mammalian glucose transporters. *Diabetes Care* 1990; **13:** 198-208.
56. James DE, Strube M, Mueckler M. Molecular cloning and characterization of an insulin-regulatable glucose transporter. *Nature* 1989; **338:** 83-87.
57. Charron MJ, Brosius FC III, Alper SL, Lodish HF. A novel glucose transporter protein expressed predominantly in insulin-responsive tissues. *Proc Natl Acad Sci USA* 1989; **86:** 2535-2539.
58. Birnbaum MJ. Identification of a novel gene encoding an insulin-responsive glucose transporter protein. *Cell* 1989; **57:** 305-315.
59. Fukumoto H, Kayano T, Buse JB, Edwards Y, Pilch PF, Bell GI. Cloning and characterisation of the major insulin-responsive glucose transporter expressed in human skeletal muscle and other insulin-responsive tissues. *J Biol Chem* 1989; **264:** 7776-7779.
60. Permutt MA, Koranyi L, Keller K, Lacy PE, Scharp DW, Mueckler M. Cloning and functional expression of a human pancreatic islet glucose-transporter cDNA. *Proc Natl Acad Sci USA* 1989; **86:** 8688-8692.
61. Li S, Baroni M, Oelbaum RS, Stocks J, Galton DJ. Association of the genetic variant of the glucose transporter with non-insulin-dependent diabetes mellitus. *Lancet* 1988; **2:** 368-370.
62. Cox NJ, Xiang K-S, Bell GI, Karam JH. Glucose transporter gene and non-insulin-dependent diabetes. *Lancet* 1988; **2:** 793-794.
63. Serjeantson SW, White B, Bell GI. Zimmet P. The glucose transporter gene and type 2 diabetes in the Pacific. In: Larkins RG, Zimmet PZ, Chrisholm DJ, eds. *Diabetes 1988.* Amsterdam: Excerpta Medica, 1989: 329-333.
64. Sivitz WI, DeSautel SL, Kayano T, Bell GI, Pessin JE. Regulation of glucose transporter messenger RNA in insulin-deficient states. *Nature* 1989; **340:** 72-74.
65. Berger J, Biswas C, Vicario P, Strout HV, Saperstein R, Pilch PF. Decreased expression of the insulin-responsive glucose transporter in diabetes and fasting. *Nature* 1989; **340:** 70-72.
66. Kahn CR, White MF. The insulin receptor and the molecular mechanism of insulin action. *J Clin Invest* 1988; **82:** 1151-1156.
67. Reddy SS, Kahn CR. Insulin resistance: a look at the role of insulin receptor kinase. *Diabetic Med* 1988; **5:** 621-629.
68. Grigorescu F, Flier JS, Kahn CR. Charaterization of binding and phosphorylation defects of erythrocyte insulin receptors in the type A syndrome of insulin resistance. *Diabetes* 1986; **35:** 127-138.
69. Grigorescu F, Herzberg V, King G *et al.* Defects in insulin binding and autophosphorylation of erythrocyte insulin receptors in patients with syndromes of severe insulin resistance and their parents. *J Clin Endocrinol Metab* 1987; **64:** 549-556.
70. Reddy SS, Lauris V, Kahn CR. Insulin receptor function in fibroblasts from patients with leprechaunism: differential alterations in binding, autophosphorylation, kinase activity, and receptor-mediated internalization. *J Clin Invest* 1988; **82:** 1359-1365.
71. Kriauciunas KM, Kahn CR, Muller-Wieland D, Reddy SSK, Taub R. Altered expression and function of the insulin receptor in a family with lipoatrophic diabetes. *J Clin Endocrinol Metab* 1988; **67:** 1284-1293.
72. Grunberger G, Comi RJ, Taylor SI, Gorden O. Tyrosine kinase activity of the insulin receptor of patients with type A extreme insulin resistance: studies with circulating mononuclear cells, and cultured lymphocytes. *J Clin Endocrinol Metab* 1984; **59:** 1152-1158.
73. Whittaker J, Zick Y, Roth J, Tayor SI. Insulin-stimulated receptor phosphorylation appears normal in cultured Epstein-Barr virus transformed lymphocyte cell lines derived from patients with extreme insulin resistance. *J Clin Endocrinol Metab* 1985; **60:** 381-386.
74. Cama A, Taylor SI. Tyrosine kinase activity of insulin receptors from an insulin-resistant patient with leprechaunism. *Diabetologia* 1987; **30:** 631-637.
75. Taylor SI, Kadowaki T, Kadowaki H, Accili D, Cama A, McKeon C. Mutations in insulin receptor gene in insulin-resistant patients. *Diabetes Care* 1990; **13**: 257-279.
76. Moller DE, Yokota A, Flier JS. Normal insulin receptor cDNA sequence in Pima Indians with NIDDM. *Diabetes* 1989; **38:** 1496-1500.
77. Flier JS, Kahn CR, Roth J. Receptors, antireceptor antibodies and mechanisms of insulin resistance. *N Engl J Med* 1979; **300:** 413-419.
78. Batarseh H, Thompson RA, Odugbesan O, Barnett AH. Insulin receptor antibodies in diabetes mellitus. *Clin Exp Immunol* 1988; **71:** 85-90.
79. Maron R, Elias D, de Jongh BM *et al.* Autoantibodies to the insulin receptor in juvenile onset insulin-dependent diabetes. *Nature* 1983; **303:** 817-818.
80. Schechter Y, Elias D, Maron R, Cohen IR. Mouse antibodies to the insulin receptor developing spontaneously as anti-idotypes I. Characterization of the antibodies. *J Biol Chem* 1984; **259:** 6411-6415.
81. Ludwig SM, Faiman C, Dean HJ. Insulin and insulin-receptor autoantibodies in children with newly-diagnosed IDDM before insulin therapy. *Diabetes* 1987; **36:** 420-425.
82. Soos MA, Siddle K. Immunological relationships between receptors for insulin and IGF-I: evidence for structural heterogeneity of IGF-I receptors involving hybrids with insulin receptors. *Biochem J* 1989; **263:** 553-563.
83. Moxham CP, Duronio V, Jacobs S. Insulin-like growth factor I receptor beta-subunit heterogeneity: evidence for hybrid tetramers composed of insulin-like growth factor I and insulin receptor heterodimers. *J Biol Chem* 1989; **264:** 13238-13244.
84. Maassen JA, Van der Zon GCM. Coupling of insulin responsive glucose transport to receptors for IGF-I in primary human fibroblasts. *Eur J Biochem* 1990; **190:** 553-557.
85. Roth J, Grunfeld C. Mechanisms of action of peptide hormones and catecholamines. In: Williams R, ed. *Textbook of Endocrinology*. Philadelphia: WB Saunders, 1982: 76-122.
86. Gorden P, Hendricks CM, Kahn CR *et al.* Hypoglycaemia associated with non-islet cell tumor and insulin-like growth factors. *N Engl J Med* 1981; **305:** 1452-1455.
87. Daughaday WH, Trevidi B, Kapadia M. Measurement of insulin-like growth factor II by a specific radioreceptor assay in serum of normal individuals, patients with abnormal growth hormone secretions and patients with tumor-associated hypoglycaemia. *J Clin Endocrinol Metab* 1981; **53:** 289-294.
88. Whittaker J, Soos M, Siddle K. Hybrid insulin receptors: molecular mechanisms of negative-dominant mutations in receptor-mediated insulin resistance. *Diabetes Care* 1990; **13:** 576-581.